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EFFECTS OF TIMBER STAND MATURITY AND MANAGEMENT ON SOME STREAM ORGANIC COMPOUNDS

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THESIS
AND
REPORTS

Final Report

for

Project #16-605-CA

Effects of timber stand maturity and management on some stream organic compounds

by

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September, 1977

INTRODUCTION

Ecosystem analysis attempts to understand the biotic community and its physical environment as a dynamic system of complementary relationships.

To illucidate these complementary relationships, measurements must be made of the energy and matter taken from the environment by the community, stored and transferred within the community, and released from the community back to the environment. Methods in the analyses of inorganic nutrient cycling are well developed and the measurements made in the analyses have played an important role in the synthesis of current hypotheses concerning the patterns observed in the structure and function of ecosystems.

Methods in the analyses of organic compound cycling are less well developed. This is unfortunate, for more information on the qualitative and quantitative nature of organic compounds and their movement within and out of the system is needed for a better understanding of the community's regulatory processes. Within the system, organics may play a large role in the movement and availability of inorganic compounds, including nutrients and toxic heavy metals. Organics may be toxins themselves, or bind toxins, or bind organic nutrient compounds such as amino acids, proteins, and carbohydrates. The identification and measurement of organics leaving the system is valuable in that these organics not only represent matter lost but also indicate the state of the system.

The type and amount of organic matter, particulate and dissolved, present in surface water has a very real effect upon the ecological status of the stream itself as well as downstream ecosystems. The effects of stream organics are varied ranging from unpleasant discoloration by "yellow organics" (Shapiro 1957, Lamar and Goerlitz 1966, Christman 1970) to inhibition of

plant growth by lignin degradation products (Borner 1955, 1956, Prill et al. 1949), fouling commercial anion-exchangers used to treat surface water (Frisch and Kunin 1960) and fish kills (McGauhey 1968).

Organic acids, both aromatic and aliphatic, are prominent in soils and surface waters as a result of plant exudates and plant tissue decomposition. The levels found are strongly linked to bacterial action because of their role in production and subsequent transformations (Wang et al. 1967). Changes in the activity of microbes will cause a shift in the level of these acids found (Stevenson 1968); however, the degree of change is a characteristic of the type of vegetational community. Gosz and Barr (1975) studied stream organics for pinon-juniper, mixed conifer, aspen, and spruce-fir communities in New Mexico. The mixed conifer association was found to contribute significantly more organic material than any other community. A major proportion of the dissolved organics were hydroxylated aromatic compounds--breakdown products of tannin and lignin.

Since the levels of organics in the drainage water of a community are a result of the plant species and rates of bacterial action the condition of plant community also is important. Over-mature or decadent stands would be expected to have increased rates of decomposition over younger, actively growing stands, therefore, the drainage water of the decadent stand should have higher levels of organics. This suggests that timber management activities which convert an over-mature stand into a young, actively growing stand should decrease the levels of organics in the drainage stream.

OBJECTIVES

The original objectives of this study were:

1) quantify natural levels of "hydroxylated aromatic" compounds (tannin,

lignin-like compounds) in streams of mixed conifer communities representing young, intermediate, and over-mature age classes.

2) Quantify changes in the levels of "hydroxylated aromatic" compounds in streams of mixed conifer communities after timber harvesting practices.

3) Use data developed in objectives 1 and 2 to help establish timber land management procedures consistent with water quality regulations.

4) To develop new techniques of organic analysis of stream waters which will illucidate the various types of organics found in Arizona and New Mexico watersheds, and the seasonal variation in organics in these watersheds.

This research was to be performed on gaged watersheds in the Apache-Sitgraves National Forest, Arizona and the Santa Fe National Forest, New Mexico. The mixed conifer communities in Arizona represent over-mature age classes. Two of the Arizona watersheds have been harvested recently and represent very young regeneration stands. The mixed conifer watersheds in the Santa Fe National Forest represent intermediate to mature communities.

The major problem encountered during the research period was a lack of stream flow in the Arizona watersheds for much of the year. This severely limited the number of collections, analyses, and the ability to detect significant differences.

Collections of stream water were made by Forest Service field technicians. Samples were collected in 500 ml polyethylene bottles (preacidified) and mailed to the University of New Mexico. Water samples were analyzed for hydroxylated aromatic compounds by the Tyrosine Method and a Hach DR colorimeter.

RESULTS

Table 1 shows results of hydroxylated aromatic compound analyses for the Arizona watersheds during the spring runoff period in 1976. There seems to

Table 1. Concentrations (mg/l) of hydroxylated aromatic compounds (expressed as tannin equivalent) in stream water of some Arizona mixed conifer watersheds

	Tannin Equivalent					
Date (1976)	North Thomas	South Thomas	Willow Creek Weir #1	Willow Creek Weir #2	East Castle	West Castle
Apr. 8	0.1	<0.1	<0.1	0.2	0.3	0.2
Apr. 9	<0.1	<0.1	0.3	0.1	0.3	0.3
Apr. 22	0.4	0.1	0.3	0.3	0.6	0.4
Apr. 23	0.2	<0.1	0.4	0.5	0.8	0.3
May 13	0.8	0.6	0.7	1.0	<0.1	0.6
May 14	0.4	0.8	1.3	0.8	0.7	1.2
June 3	0.3		<0.1			
June 5	0.1		<0.1			

be a trend toward increasing values through the spring runoff period until May, then decreasing values in June. This pattern is not correlated with stream discharge (Table 2) which shows decreasing discharge from late April through May and June. This pattern is difficult to interpret since previous work has shown that organic levels are normally related to discharge (Gosz and Barr 1975). It must be remembered that the data represent relatively few data points and conditions between the sampling points could markedly affect the results.

There are no statistically significant differences between the watersheds in terms of hydroxylated aromatic compounds even though Willow Creek #1 and West Castle watersheds were recently harvested. Comparisons with the New Mexico mixed conifer watersheds during April and early May (peak snowmelt) show that the maximum levels for the Arizona watersheds correspond well (Table 3). There are relatively large differences in hydroxylated aromatic compounds among the other vegetation types in New Mexico which corresponds with previous work (Gosz and Barr 1975).

An interesting comparison can be made between organic and inorganic analyses of water samples from the Arizona watersheds (Tables 1, 4, 5, 6, 7). In contrast to the organic data, inorganic analyses show relatively large differences among the watersheds. Calcium shows the greatest amount of difference with only South Thomas and Willow Creek #2 having mean concentrations not significantly different. For other cations the patterns are more complicated. In general, the Willow Creek watersheds have the lowest levels of Ca^{++}, Mg^{++}, Na^{+} and K^{+}, Castle Creek watersheds have the highest levels of Ca^{++} and Mg^{++}, and Thomas Creek watersheds have the highest levels of Na^{+} and K^{+}. Perhaps these data can be compared with other analyses of stream water to suggest causes.

Table 2. Gage heights (feet) at stream collection time

Date	North Thomas	South Thomas	Willow Creek Weir #1	Willow Creek Weir #2	East Castle	West Castle
Apr. 22	.364	.500	.760	.420	.490	.310
Apr. 23	.379	.528	.780	.440	.462	.304
May 13	.254	.358	.395	.410	.130	.081
May 14	.248	.350	.397	.401	.122	.08
June 3	.124		.280			
June 5	.118		.262			

Table 3. Organic levels in stream water of the Tesuque Watersheds during spring runoff

	Tannin Equivalent						
	Ponderosa Pine	Mixed Conifer	Mixed Conifer	Aspen			Spruce-Fir
Date	W-2	W-4	W-5	AW1	AW2	AW3	W-15
April 16	<0.1	1.3	0.9	<0.1	0.5	<0.1	0.4
April 20	<0.1	1.0	0.9	0.1	<0.1	<0.1	<0.1
April 26	0.4	1.1	0.7	0.2	0.2	0.1	0.3
May 3	1.6	1.3	1.1				

Table 4. Calcium concentration (ppm) of stream water collections

Date	North Thomas	South Thomas	Willow Creek* Weir #1	Willow Creek Weir #2	East Castle	West* Castle
Apr. 8	8.10	6.55	6.85	5.90	11.20	10.1
Apr. 9	8.05	6.60	6.95	6.45	11.65	9.70
Apr. 22	7.45	5.85	6.75	6.00	11.95	10.15
Apr. 23	7.75	6.10	6.75	6.10	12.15	9.95
May 13	7.50	6.00	6.70	5.50	12.80	12.15
May 14	7.50	5.95	7.30	5.50	13.00	12.20
Mean	7.72	6.18	6.88	5.91	12.12	10.71

	Willow Creek Weir #2	South Thomas	Willow Creek Weir #1	North Thomas	West Castle	East Castle
Ranked Means	5.91	6.18	6.88	7.72	10.71	12.12

Means with common underline are not significantly different ($P > .05$).

* Watershed recently harvested.

Table 5. Magnesium concentrations (ppm) of stream water collections

Date	North Thomas	South Thomas	Willow Creek* Weir #1	Willow Creek Weir #2	East Castle	West* Castle
Apr. 8	4.00	2.95	3.35	3.10	6.15	6.15
Apr. 9	4.05	3.00	3.20	3.35	6.70	5.95
Apr. 22	3.80	2.70	3.15	3.05	6.65	6.15
Apr. 23	3.80	2.70	3.20	3.15	6.85	6.00
May 13	3.85	2.80	3.30	2.90	8.00	7.45
May 14	3.85	2.85	3.75	3.05	8.25	7.80
Mean	3.89	2.83	3.32	3.1	7.1	6.58

	South Thomas	Willow Creek Weir #2	Willow Creek Weir #1	North Thomas	West Castle	East Castle
Ranked Means	2.83	3.1	3.32	3.89	6.58	7.1

Means with common underline are not significantly different ($P > .05$).

* Watershed recently harvested.

Table 6. Potassium concentrations (ppm) of stream water collections

Date (1976)	North Thomas	South Thomas	Willow Creek * Weir #1	Willow Creek Weir #2	East Castle	West * Castle
Apr. 8	0.80	0.69	0.48	0.47	0.75	0.46
Apr. 9	0.81	0.70	0.55	0.45	0.69	0.40
Apr. 22	0.88	0.74	0.54	0.45	0.71	0.57
Apr. 23	0.87	0.75	0.47	0.43	0.63	0.50
May 13	0.93	0.79	0.51	0.47	0.58	0.63
May 14	0.91	0.74	0.38	0.38	0.54	0.51
Mean	0.87	0.74	0.49	0.44	0.65	0.51

	Willow Creek Weir #2	Willow Creek Weir #1	West Castle	East Castle	South Thomas	North Thomas
Ranked Means	0.44	0.49	0.51	0.65	0.74	0.87

Means with common underline are not significantly different ($P > .05$).

* Watershed recently harvested.

Table 7. Sodium concentrations (ppm) of stream water collections

Date (1976)	North Thomas	South Thomas	Willow Creek* Weir #1	Willow Creek Weir #2	East Castle	West* Castle
Apr. 8	2.85	2.55	2.05	1.65	2.50	2.40
Apr. 9	2.85	2.55	2.00	1.80	2.55	2.35
Apr. 22	3.00	2.55	2.20	1.85	2.65	2.60
Apr. 23	2.95	2.55	2.20	1.90	2.50	2.50
May 13	2.96	2.62	2.30	1.87	2.96	2.74
May 14	2.97	2.63	2.23	1.80	2.96	2.67
Mean	2.93	2.58	2.16	1.81	2.69	2.54

	Willow Creek Weir #2	Willow Creek Weir #1	West Castle	South Thomas	East Castle	North Thomas
Ranked Means	1.81	2.16	2.54	2.58	2.69	2.93

Means with common underline are not significantly different ($P > .05$).

* Watershed recently harvested.

DEVELOPMENT OF NEW TECHNIQUES AND METHODS

A complicating problem to the results of organic compounds reported above is that our current research shows that the current techniques of stream organics analysis are quantitatively and qualitatively inaccurate. Freeze concentration with subsequent thin layer chromatography involves time and indeterminable error. Colorimetric methods, such as the Tyrosine Method using the Hach DR Colorimeter, are simple and fast but only measure the capacity of the stream organics, defined as "aromatic hydroxyls", to reduce the tyrosine reagent. This is an indirect, inaccurate, and non-qualitative analysis of stream organics. The colorimetric methods do not qualify or quantify the individual organic compounds which are dissimilar chemically, and function differently with respect to their reactivity and specificity with the other compounds in the system. Concentration and separation methods must be developed so that the individual organics can be identified and quantified. Subsequent determination of their activities with the other compounds and ions in the system and then following the qualitative and quantitative changes in these organics through the seasons should provide more sensitive, valuable information relevant to the state of an ecosystem than the colorimetric methods can provide.

A goal which evolved during the period of this study was to develop techniques for the separation of stream organics. High pressure liquid chromatography was used because it provides fast and quantitatively sensitive separation of the organics. The reverse phase mode was chosen for this purpose because the column support has a high affinity for relatively non-polar polymeric compounds. Polyphenolic compounds, which are relatively non-polar polymeric compounds, are reported to constitute the majority of the organics

in stream water. As the polar mobile phase (stream water) passes over the support, these organics are adsorbed to the support's surface. In this way the organics can be concentrated on the column. Trace enrichment of the organics in this way allows reduction of the lower limits of detection to parts per trillion. This technique will be valuable at a time when the concentration of organics in stream water is at a minimum. This method also eliminates extensive sample preparation. The organics that are concentrated on the column can then be eluted off by the use of solvent systems of decreasing polarity. With the use of other columns and solvent systems, most of the other types of organics in stream water can be concentrated and separated.

The first task was to find the minimum amount of water sample necessary for adequate concentration of the organics on the column. The second task was to find the most efficient solvent system for the separation of the stream organics and the maximum flow rate that will maintain the separation. The result should be separation of the organics in the least time possible using the least amount of sample.

MATERIALS AND METHODS

A Water's Associates, Inc. high pressure liquid chromatographic system was used. A Model 6000 A Solvent Delivery System was used in conjunction with a Model 450 Variable Wavelength Detector. Flow rates of 0.5 to 2.0 ml/min. were used, although the system was capable of 9.9 ml/min. The detector was set at 254 nm. The column used was a Bondapak μC18, 4mmID x 30cm. The packing in this column consisted of a monomolecular layer of octadecyl-trichloro-silane chemically bonded to 10μ silica beads.

The solvent system used was acetonitrile versus water (Figure 1). The

acetonitrile was filtered with millipore 0.5μ organic filters. The water was doubled distilled, made to 1% acetic acid, then filtered with millipore 0.5μ aqueous filters. Degassing was necessary upon mixing the acetonitrile and water to prevent bubble formation in the solvent lines.

500 mls of watershed 4 (W-4) stream water were collected in nalgene bottles. All air was expelled from the bottles. The samples were kept at 5°C during transport and storage. Samples were analyzed 1 to 3 days after collection. 25 to 100 mls of sample were filtered to 0.5μ, made to 1% acetic acid which protonates the individual organics to one form, and then fed into the solvent lines. The organics deposited on the column were then eluted off with various concentrations of acetonitrile to water. Elutions were carried out at approximately 70°C. 0.5 ppm tannic acid in water was used as a standard (Figure 1).

RESULTS AND DISCUSSION

The amount of water sample needed in the enrichment process depended on the impurities in the solvent rather than on the sensitivity of the instrument. Fifty mls of water sample was determined to be the least amount necessary for enrichment. The maximum flow rate that allowed complete separation of the five major peaks was determined to be 2 ml/min. The solvent systems were stepwise gradients of increasing acetonitrile concentration. The gradient of run 20 (Figure 2) achieved the best separation.

Tannic acid was run with the same solvent system and flow rate as run 20 to establish a rough idea of the molecular weights and polarity of the five peaks. Peak 3 (Figure 1), the center peak, had the same retention time as tannic acid (M.W. ca. 1740).

To determine changes in the qualitative and quantitative nature of the

organics due to sample storage, a sample was analyzed 13 days after collection (Figure 3). This analysis was compared to the analysis of a sample collected a week later but analyzed 2 days after collection (Figure 4). The major peaks did not appear to change. However, the separation at this point needed greater refinement. It is possible that changes were taking place that were not recognizable at this level of separation. Therefore, subsequent analyses were run 1 to 2 days after collection of sample to minimize possible changes. Length of storage without appreciable changes in the organics should be determined once complete resolution is achieved.

The next step in this procedure and one which we are currently working on, is the fraction collection of the five peaks, then individual runs with each fraction using different solvent systems, flow rates, and recycling procedures. Once this is accomplished, the stream organics will be identified through spectral and molecular weight analyses. After this, the reactivities of these stream organics with proteins, carbohydrates, inorganic ions, and heavy metals will be determined. These data, in addition to following quantitative changes of the stream organics through the seasons, will provide meaningful, comparable information on the state changes of the watershed.

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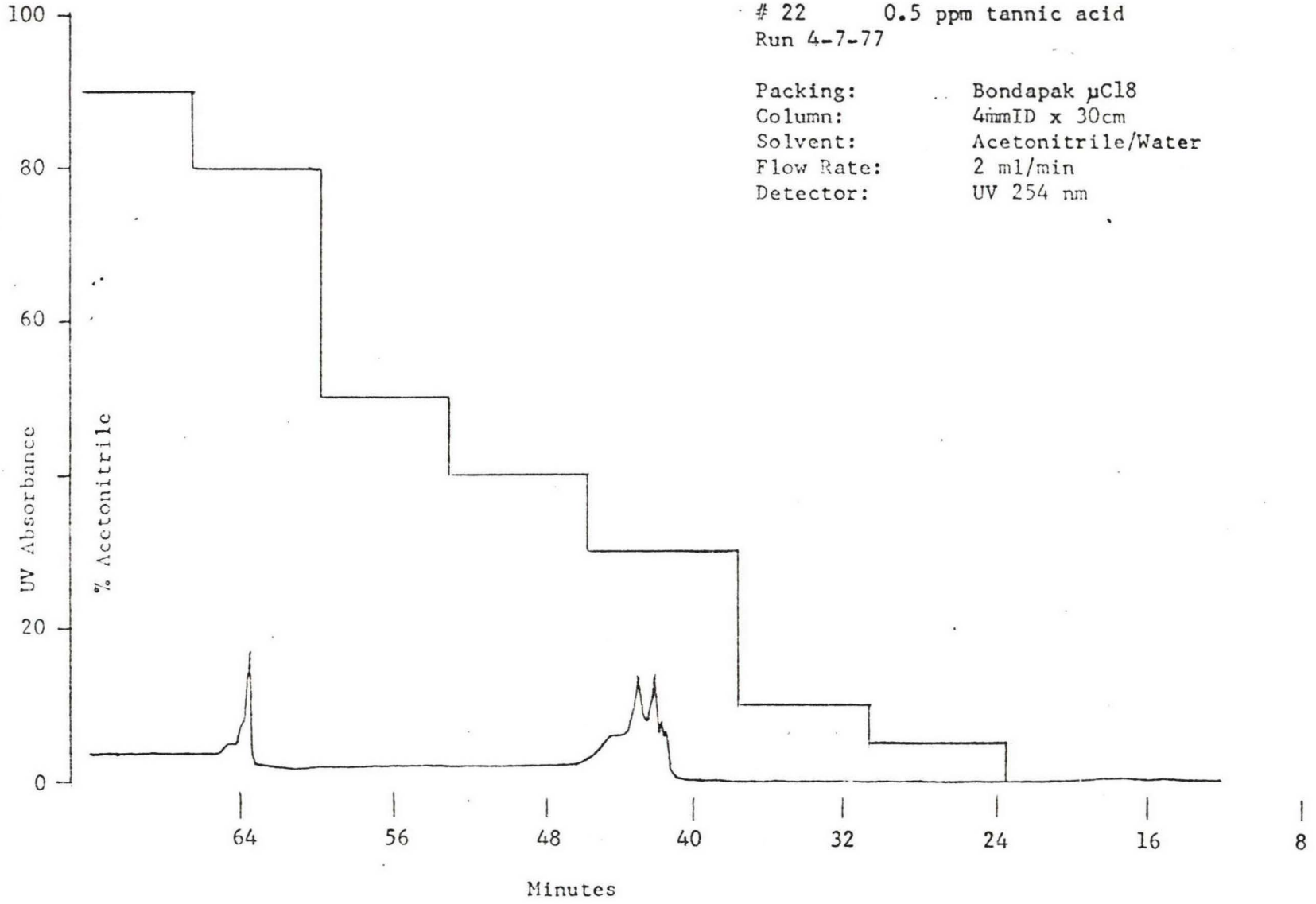

Figure 1. Tannic acid retention time using reverse phase LC

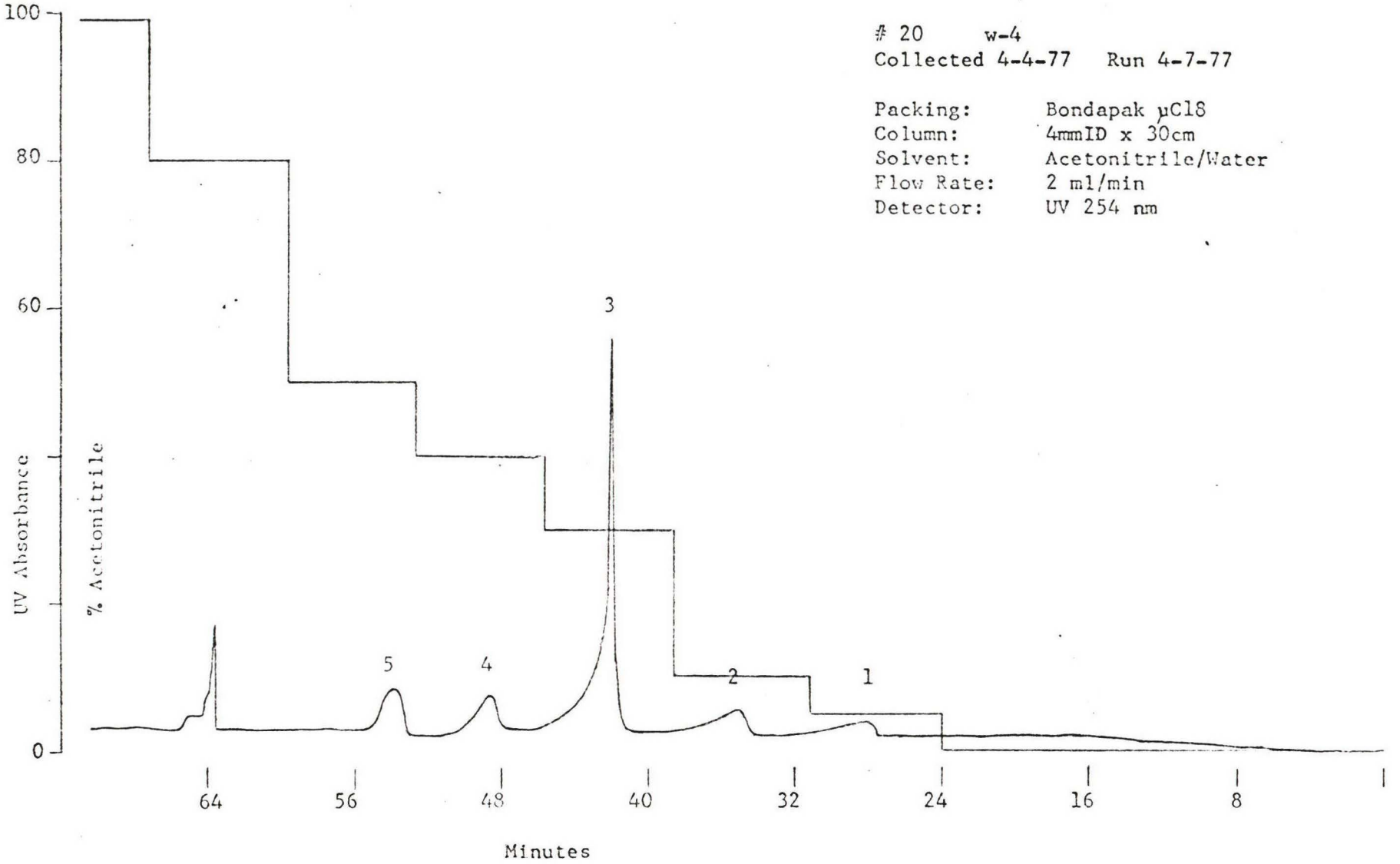

Figure 2. Reverse phase LC separation of watershed 4 stream organics.

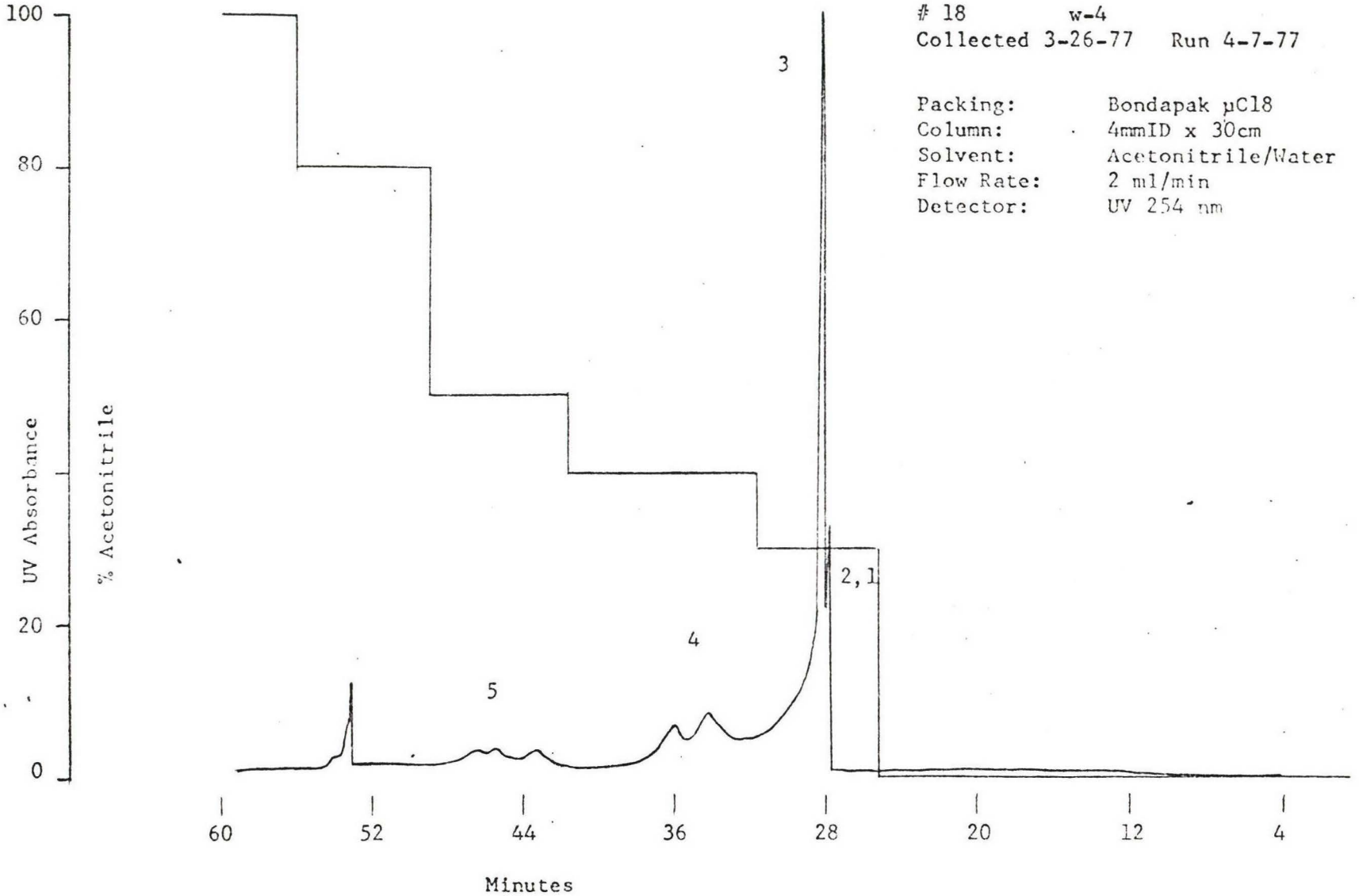

Figure 3. Reverse phase LC analysis w-4 stream organics afters 13 days sample storage.

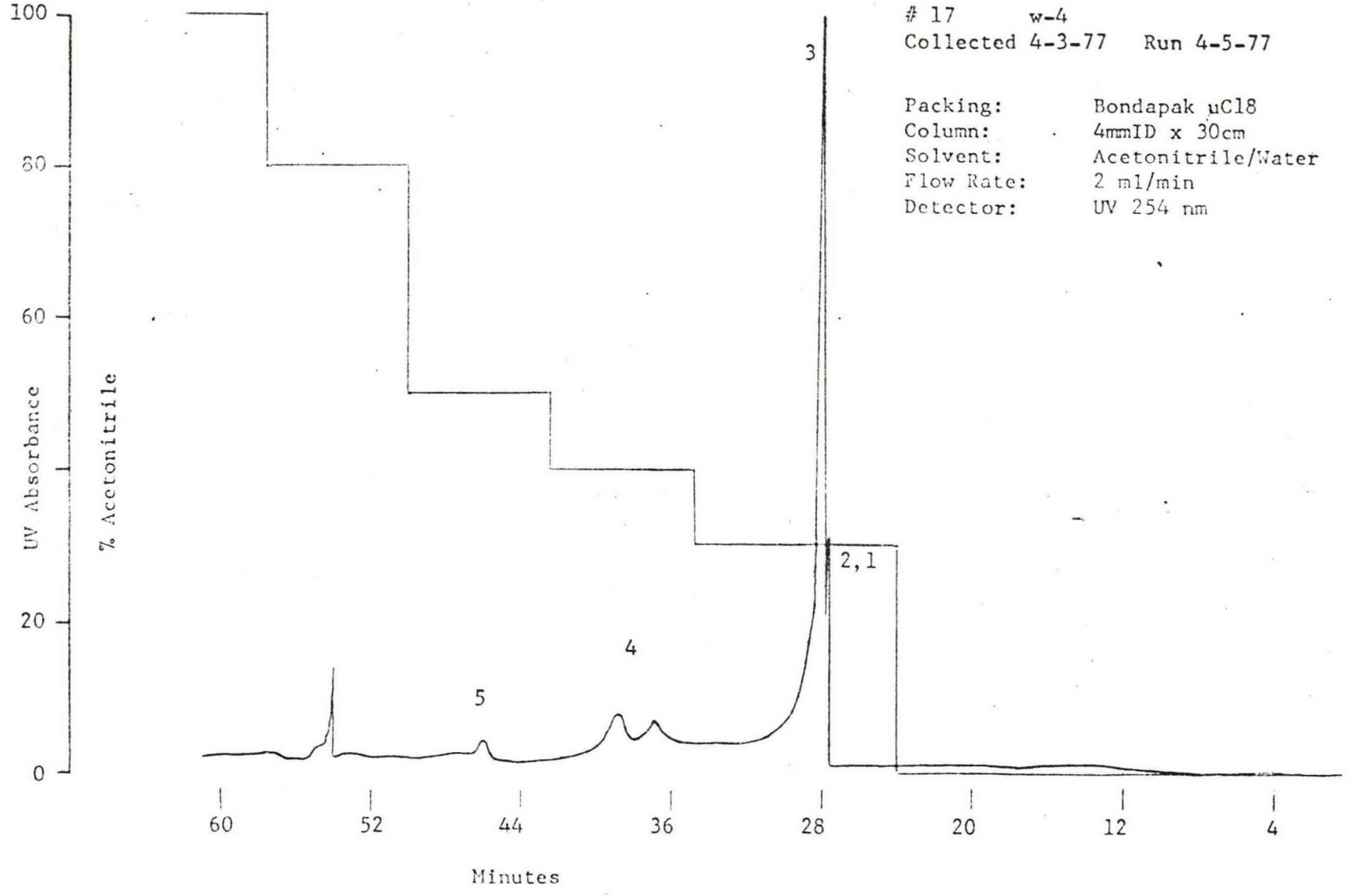

Figure 4. Reverse phase LC analysis w-4 stream organics after 2 days sample storage.